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Research Article

Simultaneous determination of bromine and iodine species in dairy products by LC-ICP-MS

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Abstract

This study developed and optimized a liquid chromatography hyphenated with inductively coupled plasma mass spectrometry (LC-ICP-MS) method for the simultaneous determination of iodine (iodide and iodate) and bromine species (bromide and bromate) in dairy products. Samples were extracted with a 10 mM ammonium carbonate solution containing 0.5 mM Mg-EDTA, then analyzed by LC–ICP–MS using an anion-exchange column (Shodex NI-424, 5 µm × 4.6 mm × 150 mm) and a mobile phase of 50 mM ammonium carbonate containing 1% methanol (pH = 10). The limits of quantification (LOQs) for iodate, iodide, bromate, and bromide in liquid milk matrices were established at 1.10, 1.77, 8.50, and 12.2 µg/100g, respectively. The corresponding values for solid samples were found to be 5.50, 8.90, 42.5, and 61.0 µg/100g. The method repeatability ranged from 1.90% to 6.01%, with recoveries between 86.3% and 109.8%, meeting AOAC performance criteria. Analysis of 26 commercial milk samples from Hanoi showed no detectable iodate or bromate. Iodide concentrations were highest in powdered milk (13.5 – 203 µg/100g) and lowest in liquid milk (1.29–34.4 µg/100g). Bromide was detected in most products, with the highest levels in powdered milk (996–2229 µg/100g), followed by liquid milk (5.60 – 397 µg/100g) and yogurt (259 – 383 µg/100g). The results confirm that LC-ICP-MS is a robust and reliable technique for the simultaneous determination of iodine and bromine species in dairy products, providing essential data for nutritional evaluation and food safety assessment.

Keywords: *iodate, iodide, bromate, bromide, LC-ICP-MS, dairy products.*

1. INTRODUCTION

Milk and dairy products are essential food sources that provide proteins, lipids, carbohydrates, vitamins, minerals, and numerous micronutrients important for human health, particularly for children and pregnant women. In addition to major nutritional components, milk may also contain various trace elements, among which iodine (I) is an essential nutrient involved in the synthesis of thyroid hormones and plays a critical role in physical and cognitive development [1]. In the diet, iodine is commonly supplemented through iodized salt (iodate or iodide) and fortified foods [2]. However, the iodine content in milk can fluctuate depending on livestock feed, farming environment, and processing conditions [3]. In contrast, bromine (Br) is not an essential element; bromide can exert neurotoxic and thyroid-disrupting effects when accumulated at high levels, while bromate is classified as a potential carcinogen [4]. Bromine contamination may originate from the environment, food additives, or manufacturing practices [5]. The presence of bromine in dairy products can interfere with and reduce iodine absorption, consequently increasing the risk of iodine deficiency, hypothyroidism, and

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associated adverse health effects [5]. Therefore, the simultaneous monitoring of iodine and bromine in milk is scientifically meaningful for nutritional and toxicological studies and is practically important for food quality control and public health protection.

Globally, numerous studies have focused on the analysis of iodine and bromine species in food matrices. Basumallick and Rohrer employed ion chromatography with pulsed amperometric detection to quantify iodide and iodate in infant formula and soy milk [6], whereas Niemann *et al.* applied PAD detection for iodide and thiocyanate in powdered milk [7]. Sanchez and Szpunar developed an SEC-ICP-MS method to investigate iodine species in milk [8], while Nelson and colleagues utilized LC-ICP-MS for the simultaneous determination of iodide, iodate, bromide, and bromate in infant formula [9]. In addition, other analytical approaches-such as ion chromatography with UV-Vis and conductivity detection [10], LC-ICP-MS [11], ICP-MS [12], capillary electrophoresis [13–14], and CE-ICP-MS [15]-have also been developed for analyzing various iodine and bromine species in different sample matrices including water, seaweed, seafood, and table salt, demonstrating the diversity of analytical strategies available for iodine and bromine speciation.

However, the simultaneous determination of bromine and iodine species in milk remains challenging. The primary difficulties arise from the chemical characteristics of bromine- and iodine-containing species in a complex matrix rich in proteins, carbohydrates, lipids, and minerals, which can affect extraction efficiency and promote redox transformations. These factors make conventional techniques such as ion chromatography, liquid chromatography, or capillary electrophoresis insufficient for reliable trace-level analysis. Therefore, it is necessary to employ advanced analytical methods with high sensitivity, efficient separation, and strong resolving capability to obtain accurate and practically meaningful results [16, 17]. Liquid chromatography coupled with inductively coupled plasma–mass spectrometry (LC-ICP-MS) is a modern technique that offers several advantages: LC enables chromatographic separation of elemental species, while ICP-MS provides high sensitivity, low detection limits, and a wide linear range [18]. This combination allows accurate simultaneous quantification of iodine and bromine species and delivers valuable speciation information, thereby supporting more comprehensive assessments of nutritional value and toxicity.

Considering the points outlined above, the study entitled "Simultaneous determination of selected bromine and iodine species in dairy products using LC-ICP-MS" was conducted with the objective of developing and optimizing an analytical procedure for the simultaneous determination of iodate, iodide, bromate, and bromide in milk using LC-ICP-MS. The method was subsequently applied to analyze and assess the current status of these species in various commercially available dairy products.

2. MATERIALS AND METHODS

2.1. Study subjects

The targets of this study were iodine species (iodate, iodide) and bromine species (bromide, bromate) present in dairy products, including powdered milk, yogurt, and liquid milk, collected from retail stores and supermarkets in Hanoi, Vietnam.

2.2. Instruments and equipment

A Flexar liquid chromatography system coupled with a Nexion 350X ICP-MS (PerkinElmer) and an anion-exchange column NI 424 (5 µm × 4.6 mm × 150 mm, Shodex) was used in this study. Samples were weighed using an analytical balance (Mettler Toledo) with an accuracy of 0.0001 g. A horizontal shaker SA300 (Yamato) and a centrifuge (Hermle) were employed for extraction and phase separation of analytes from the sample matrix. A 0.45 µm membrane filter (Minisart) was used for sample filtration prior to analysis.

2.3. Chemicals and standards

In this study, single-component standards of iodate, iodide, bromate, and bromide at concentrations of 1000 ppm (Merck) were used. Other chemicals, including ammonium carbonate (>99%), Mg-EDTA (>99.9%), methanol (>99.9%), ammonia solution (25%), and sodium hydroxide (99%), were of analytical grade and supplied by Merck. Deionized water was used throughout the experiments. Argon gas with a purity of 99.999% (Messer) was employed. In addition, instrument calibration solutions (PerkinElmer) were also utilized.

2.4. Mobile phase, extraction solvents, and working standard solutions

2.4.1. Mobile phase: 50 mM ammonium carbonate solution containing 1% methanol, adjusted to pH 10

Approximately 4.8 g of ammonium carbonate was accurately weighed into a beaker, dissolved in deionized water, and transferred to a 1000 mL volumetric flask. Deionized water was added to a volume of approximately 800 mL. The pH was adjusted to 10 by the gradual addition of 10% ammonia solution. Subsequently, 10 mL of methanol was added, and the solution was brought to volume with deionized water. The mixture was then filtered through a membrane filter and sonicated to remove dissolved gases before being used as the mobile phase. The mobile phase was freshly prepared on a daily basis.

2.4.2. Extraction solvent: 10 mM ammonium carbonate solution containing 0.5 mM Mg-EDTA.

Approximately 0.961 g of ammonium carbonate and 0.186 g of Mg-EDTA were accurately weighed into a beaker, dissolved in deionized water, and transferred to a volumetric flask. The solution was then made up to 1000 mL with deionized water.

2.4.3. Working standard solutions

From the initial 1000 ppm stock standard solutions, appropriate dilutions were prepared in deionized water to obtain intermediate standards of iodate, iodide, bromate, and bromide at concentrations of 1 ppm and 10 ppm. These intermediate standards were further diluted with deionized water to produce working standards at concentrations of 2, 5, 10, 20, 40, 80, and 100 ppb. The working standard solutions were freshly prepared each day prior to analysis.

2.5. Research methods

2.5.1. LC-ICP-MS analytical method

Optimization of liquid chromatography (LC) conditions: The liquid chromatography (LC) conditions were investigated to address issues related to the elution capability and resolution of the analytes within the analysis time. The parameters examined included: the type of anion-exchange separation column (Shodex NI424, Hamilton PRP-X100, and Zirchrom columns) [19–21]; the type of mobile-phase solvent (ammonium carbonate, ammonium nitrate, and ammonium acetate) [21, 22]; the mobile-phase concentration (25, 50, and 75 mM) and composition (with the addition of varying volumes of methanol from 1–3%); and the gradient flow-rate program of the mobile phase.

Optimization of ICP-MS conditions: In addition to the instrumental parameters affecting measurement sensitivity-such as carrier gas flow rate, plasma power, nebulizer gas flow rate, and plasma torch depth-which were automatically optimized according to the instrument's operating guidelines, the selection of mass-to-charge ratios for iodine and bromine was investigated to ensure that the chosen isotopes were abundant and minimally influenced by polyatomic interferences. Furthermore, the helium collision gas flow rate was also optimized to reduce spectral interferences.

2.5.2. Sampling, preservation and sample preparation

Milk product samples were randomly collected within the Hanoi area. Powdered milk and commercially packaged liquid milk were stored in a dry place at ambient temperature, whereas yogurt and pasteurized fresh milk samples were kept refrigerated at 2–8°C prior to homogenization for analysis. For sample preparation, approximately 1 g of solid samples and 5 g of liquid samples were weighed accurately, followed by the addition of 30 mL of extraction solvent. The extraction solvents investigated included: deionized water, 5% acetic acid, 0.5% nitric acid, 0.1% NaOH, 5% ammonia, 10 mM ammonium acetate containing 0.5 mM Mg-EDTA, and 10 mM ammonium carbonate containing 0.5 mM Mg-EDTA. Extraction of analytes from the matrix was performed using either ultrasonic agitation or horizontal shaking (with optimization of extraction technique and extraction time). The resulting mixture was centrifuged, and the supernatant was transferred to a 50 mL volumetric flask and diluted to volume with the extraction solvent. The solution was then filtered through a 0.45 µm membrane filter and analyzed using LC-ICP-MS.

2.5.3. Data processing method

Analytical results for the target compounds were processed using Chromera software. Method validation data were evaluated using Microsoft Excel 2019.

3. RESULTS AND DISCUSSION

3.1. Optimization of LC-ICP-MS conditions for the simultaneous determination of iodine and bromine species

3.1.1. Results of column selection studies

Calibration solutions were used for automatic optimization of the ICP-MS detector, and the key instrumental parameters obtained are presented in **Table 1**.

Table 1. *ICP-MS instrument parameters*

Parameter	Value
RF power	1600 W
Plasma gas flow rate	12 L/min
Auxiliary gas flow rate	1.2 L/min
Nebulizer gas flow rate	0.82 L/min
Plasma torch depth	8 mm
Dwell time	250 ms

On this basis, several anion-exchange columns were selected for evaluation of their retention and elution performance for the analytes under the investigated mobile-phase conditions, consisting of 50 mM ammonium carbonate containing 1% methanol at pH 10.

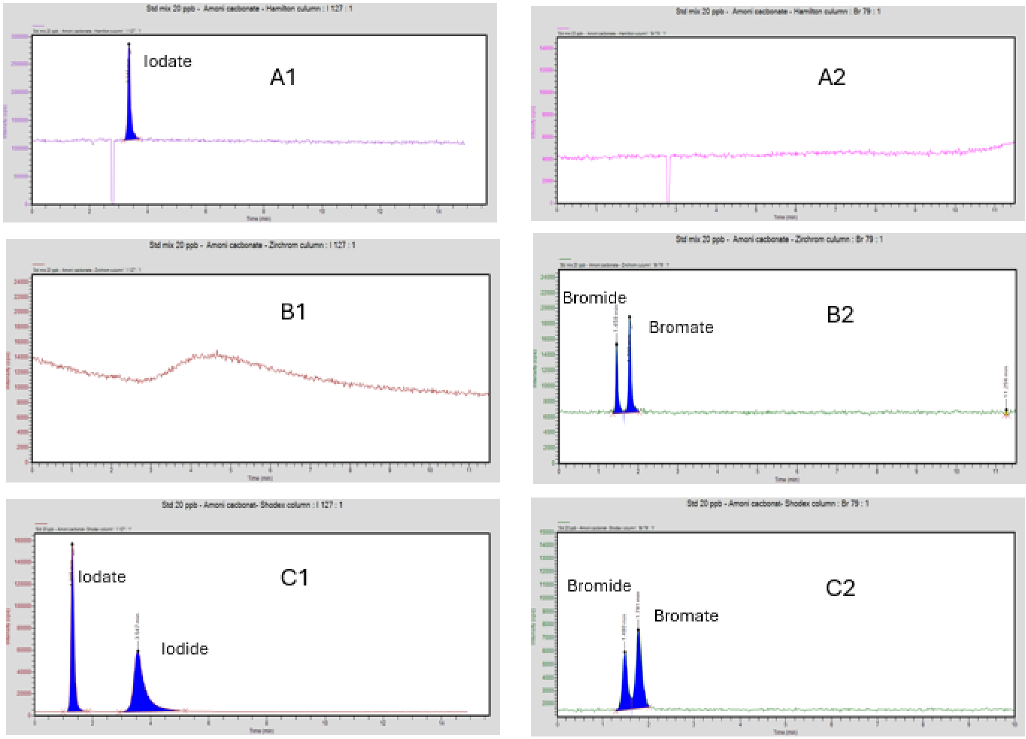

Figure 1. *Results of column selection experiments (A – Hamilton PRP X-100 column, B – Zirchrom column, C – Shodex NI424 column)*

The results showed that when the Hamilton PRP-X100 column (**Figure 1-A1&A2**) and the Zirchrom column (**Figure 1-B1&B2**) were used, only one of the two species-iodate (**Figure 1-A1**) or bromate and bromide (**Figure 1-B2**)-could be detected and analyzed within the desired analysis time of 10 min. Under the same conditions, the Shodex column (**Figure 1-C1&C2**), which is based on a poly(hydroxymethacrylate) gel matrix functionalized with quaternary ammonium groups, exhibited the capability to simultaneously separate and quantify all four analytes (iodate, iodide, bromate, and bromide). Therefore, the Shodex NI424 column (5 µm × 4.6 mm × 150 mm) was selected for subsequent method optimization and investigations.

3.1.2. Results of mobile phase solvent and pH optimization

In LC-ICP-MS analysis, the mobile phase must meet not only purity requirements but also several analytical criteria, including volatility to avoid residue formation and elevated background signals in the plasma (thereby preventing the introduction of ions such as Na^+, K^+, phosphate, or sulfate), low formation of polyatomic interferences, chromatographic compatibility, and sufficient ionic strength to separate the iodine and bromine species. On this basis, several salts were selected for evaluation as mobile phases, including ammonium acetate, ammonium nitrate, and ammonium carbonate, each at a concentration of 10 mM, adjusted to pH 10 and prepared in solutions containing 1% methanol.

The results indicated that ammonium nitrate exhibited strong elution capability (**Figure 2-A1&A2**); however, this led to overlap between the bromide and bromate peaks, and modifications of the mobile phase-particularly pH reduction-did not produce a meaningful improvement in their resolution. In contrast, the ammonium acetate mobile phase (**Figure 2-B1&B2**) showed weaker elution strength, resulting in strong retention of iodide on the column; under a 10 min chromatographic window, iodide was not eluted, and only the iodate peak was observed (**Figure 2-B1**).

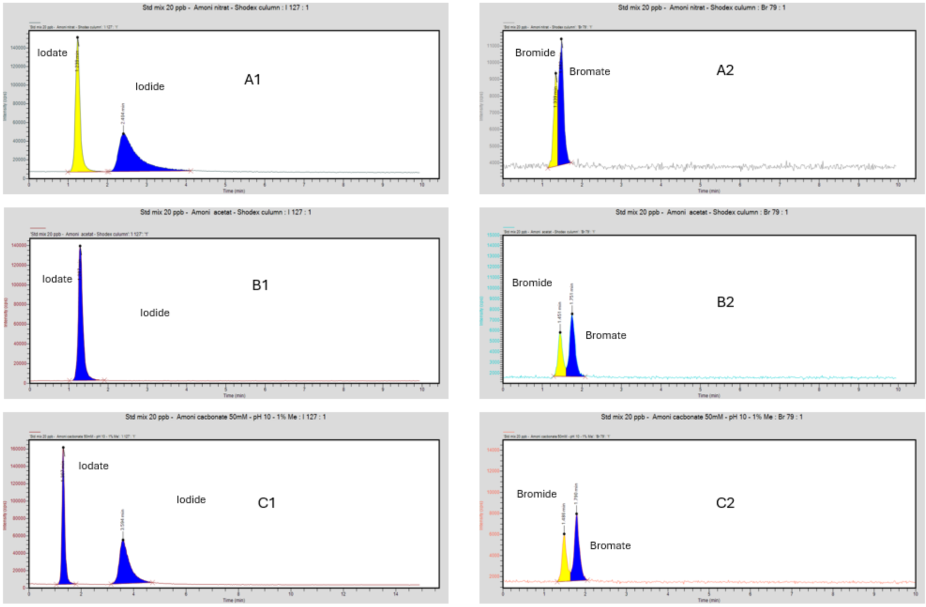

Figure 2. *Results of mobile phase solvent selection (A – ammonium nitrate, B – ammonium acetate, C – ammonium carbonate) at 10 mM concentration*

Meanwhile, the ammonium carbonate mobile phase demonstrated good separation efficiency for the target analyte anions within a 10 min timeframe. This performance fully satisfies practical analytical requirements, ensuring adequate sensitivity and resolution while saving time and reducing consumable costs.

The study also conducted an investigation under the same ammonium carbonate mobile phase conditions, but with the pH reduced by one unit (pH = 9). In this scenario, the retention time of the iodide species was extended to over 10 min, and concurrently, the resolution between bromate and bromide did not improve (**Figure 3**). Consequently, the study did not proceed with further investigation of lower pH values.

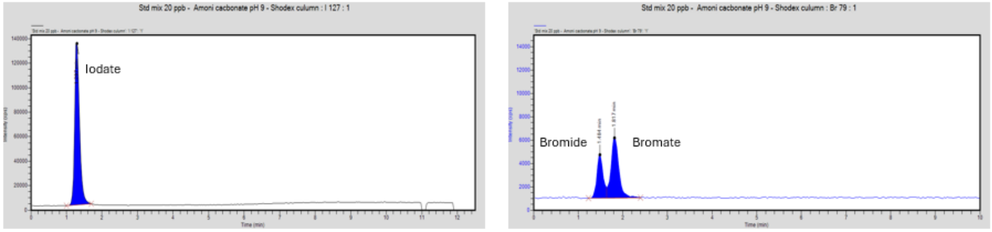

Figure 3. *Investigation of the 10 mM ammonium carbonate mobile phase with 1% MeOH, pH 9*

In addition, at the evaluated pH of 10 (**Figure 2-C1&C2**), the retention times of the anions were appropriate; increasing the pH resulted in reduced resolution between iodate and iodide and could also adversely affect the functional groups on the column's stationary-phase material. Therefore, the mobile phase was prepared at pH 10, and subsequent investigations focused on other parameters-such as mobile-phase concentration, composition, and gradient conditions-to achieve adequate sensitivity and resolution for all target ions.

3.1.3. Investigation and optimization of mobile phase composition and concentration

The effects of other mobile-phase components, including the addition of methanol and variations in salt concentration, were investigated to enhance the separation efficiency and sensitivity of the analyte anions, particularly bromate and bromide. The concentration of ammonium carbonate was varied between 25 and 75 mM, while methanol was added at levels of 1–3% (v/v). The results showed that no substantial differences were observed in the chromatographic separation of the bromine species under either condition. However, increasing the proportion of methanol led to a noticeable decrease in the signal intensity of both bromide and bromate (**Figure 4**). To maintain stable iodine and bromine signals in the milk matrix, which contains a high level of organic compounds, and to improve peak shape (sharper peaks with reduced tailing) while simultaneously minimizing the salt load introduced into the plasma, the methanol content in the mobile phase was set at 1%, and the ammonium carbonate concentration was fixed at 50 mM.

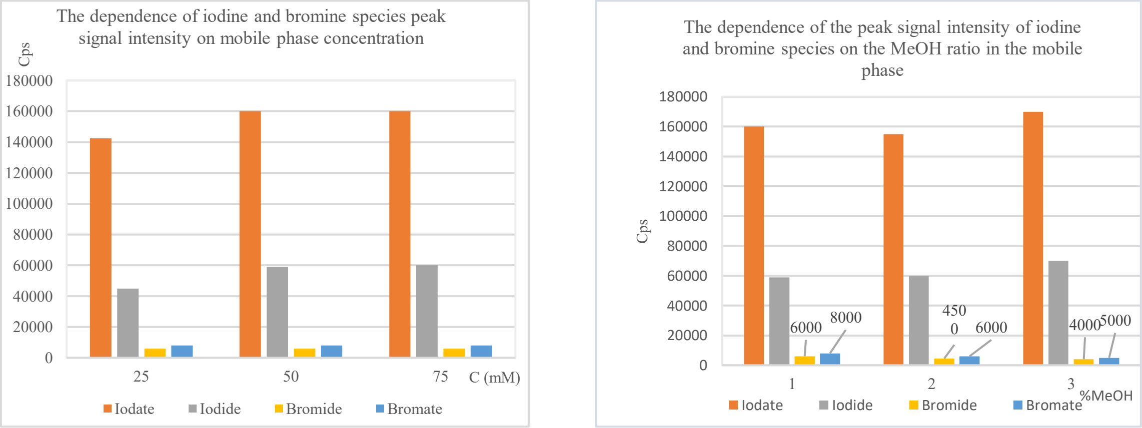

Figure 4. *Investigation of the dependence of iodine and bromine species signal intensity on mobile phase salt concentration and MeOH ratio*

3.1.4. Optimization of mobile phase flow rate gradient

Under the investigated conditions, satisfactory resolution was obtained for iodate and iodide; however, the peaks of bromate and bromide were not fully separated in several cases. To address this co-elution issue, a series of flow-rate gradient elution programs was evaluated. The results indicated that applying a flow-gradient program significantly improved the resolution between bromate and bromide without increasing the overall analysis time. The optimized gradient program was established as follows: the initial flow rate (TF) was set at 0.4 mL/min; held at 0.4 mL/min from 0–4 min; increased to 0.75 mL/min from 4–7 min; and subsequently returned to 0.4 mL/min from 7–10.5 min. The chromatogram obtained under these optimized conditions clearly demonstrates the enhanced separation of the analyte peaks (**Figure 5**).

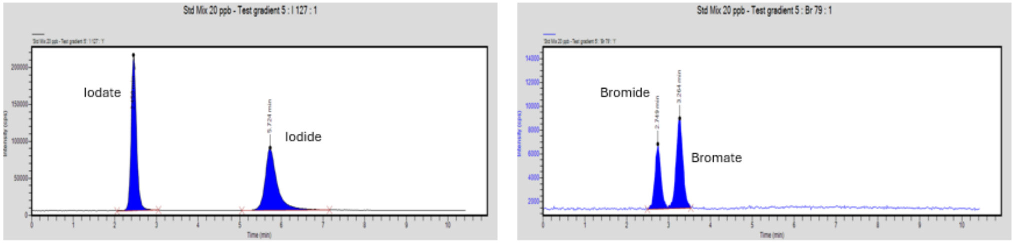

Figure 5. *Chromatogram of iodine and bromine species under optimized gradient conditions*

3.1.5. Optimization of the Kinetic Energy Discrimination (KED) mode

Based on their natural abundance and minimal spectral interferences, ^{127}I and ^{79}Br were selected as the isotopes for iodine and bromine analysis. When operating in kinetic energy discrimination (KED) mode to mitigate interferences, large polyatomic ions with low kinetic energy undergo more frequent collisions, which effectively reduces the signal of interfering species. This is particularly crucial in the case of ^{79}Br, where strong interference from Ar/K (^{40}Ar + ^{39}K → m/z 79) is prevalent due to their abundance in the sample matrix and plasma gas.

Interference removal was investigated by incrementally increasing the helium (He) gas flow rate at levels of 0.5, 1.0, 1.5, 2.0, and 2.5 mL/min (**Figure 6**). The results demonstrated that as the He gas flow rate increased, both the background interference signal and the analyte signal decreased. This indicates that while the selectivity of the method improves, the sensitivity is concurrently reduced.

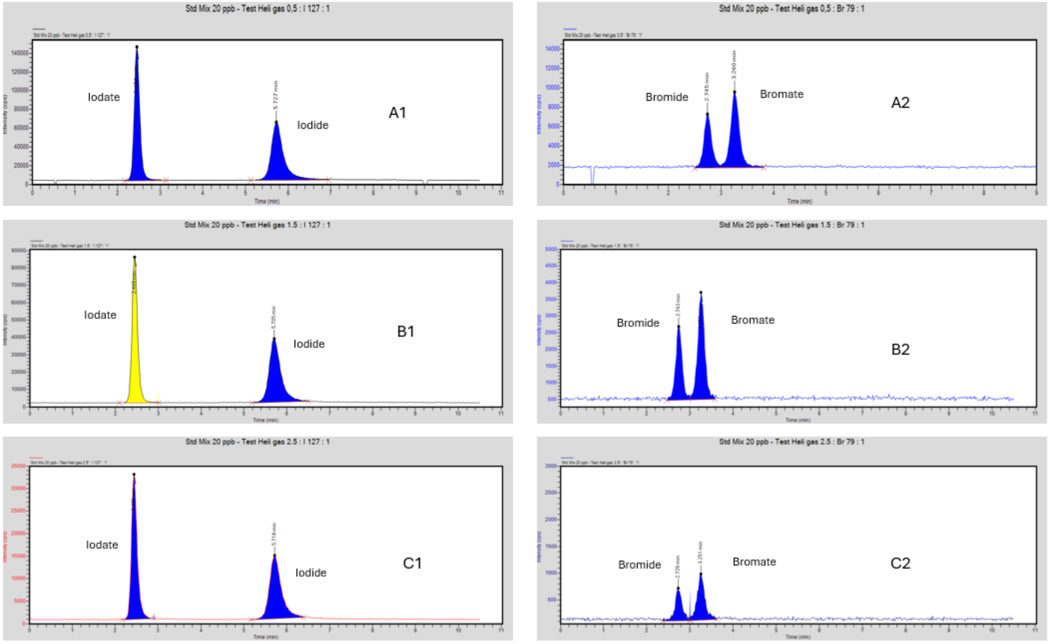

Figure 6. *Results of the investigation of helium gas flow rates at 0.5 mL/min (A), 1.5 mL/min (B), and 2.5 mL/min (C)*

Milk matrices are typically rich in mineral elements, particularly potassium, which can generate severe interferences through its combination with argon. Therefore, the use of He collision gas must be carefully evaluated to ensure effective interference removal while maintaining adequate analytical sensitivity for the target elements. Accordingly, a He flow rate of 1.5 mL/min was selected for the determination of iodine and bromine.

3.2. Investigation and optimization of the sample preparation procedure

3.2.1. Optimization of extraction solvent selection

The study investigated the selection of several extraction solvents, including: deionized water, 5% acetic acid, 0.5% nitric acid, 0.1% NaOH, 5% ammonia, 10 mM ammonium acetate containing 0.5 mM Mg-EDTA, and 10 mM ammonium carbonate containing 0.5 mM Mg-EDTA.

Deionized water (**Figure 7-A1&A2**) is a simple solvent that easily dissolves anions; however, its efficiency in releasing analytes from the protein-lipid matrix is limited. Furthermore, iodate species are easily reduced to iodide by reducing agents present in milk, such as vitamin C, sulfites, and proteins containing –SH groups [23, 24]. Conversely, dilute acids such as 5% acetic acid and 0.5% nitric acid can completely digest the sample matrix and yield high iodine recovery. Nonetheless, they cause the transformation of the original iodate species (100% conversion to iodide or loss, **Figure 7-B1&B2**) [23, 25], and are therefore typically only suitable for the determination of total iodine content.

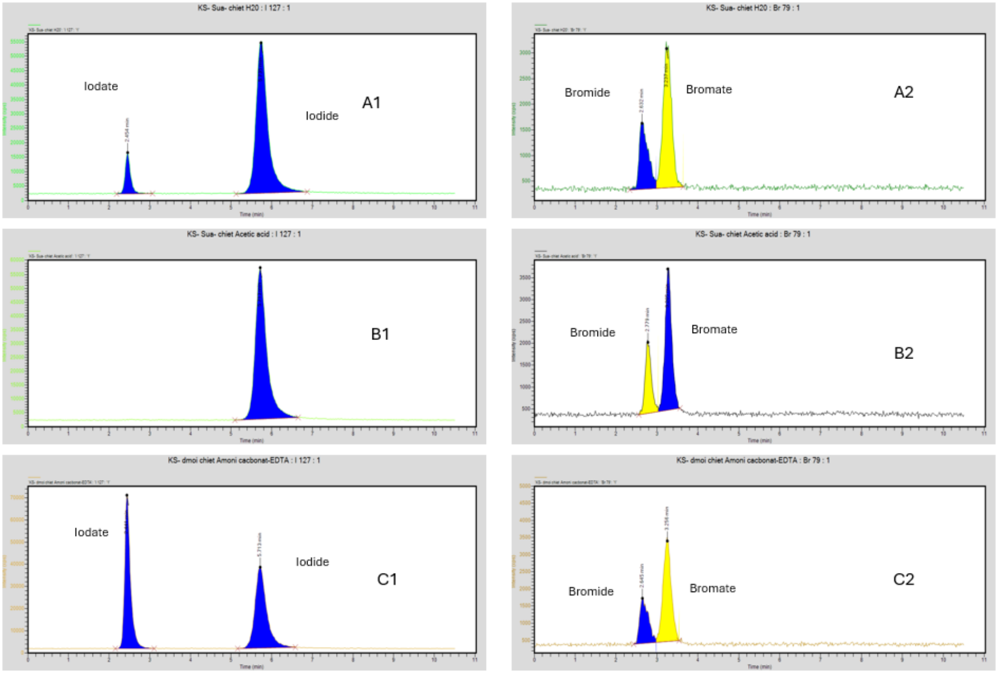

Figure 7. *Results of extraction solvent selection investigation (deionized water – A, acetic acid – B, ammonium carbonate and Mg-EDTA – C)*

Alkaline buffer solutions such as 0.1% NaOH, 5% ammonia, 10 mM ammonium carbonate, and 10 mM ammonium acetate exhibited improved recoveries of iodate species, ranging from 65.0 to 86.6%. Among these, the highest recoveries were obtained using 10 mM ammonium carbonate containing 0.5 mM Mg–EDTA as the extraction solvent (**Figure 7-C1&C2**). This solvent not only provides compatibility with ICP-MS mobile phases but also offers sufficient ionic strength to facilitate the release of iodine and bromine species from the sample matrix. Additionally, the mildly alkaline pH of the carbonate buffer helps stabilize iodate [17]. The inclusion of a mild complexing agent such as Mg–EDTA at low concentration further prevents reduction processes and promotes the release of protein-bound or metal-associated iodine in milk [26].

3.2.2. Optimization of sample extraction time and technique

During the preparation of milk powder samples for the simultaneous determination of iodide and iodate, the use of prolonged ultrasonication or elevated temperatures is generally discouraged when preservation of the chemical species is required. Both ultrasonic agitation and extended heating can promote the release of reducing substances from the protein–lipid matrix and accelerate redox reactions, thereby facilitating the conversion of iodate to iodide and ultimately biasing speciation results [27, 28]. In addition, ultrasonication may disrupt the sample matrix structure and solubilize a wide range of organic compounds, leading to increased matrix complexity and adversely affecting ICP-MS signal quality [29]. **Figure 8** illustrates the influence of ultrasonication time in water at ambient temperature on the recoveries of the target anions.

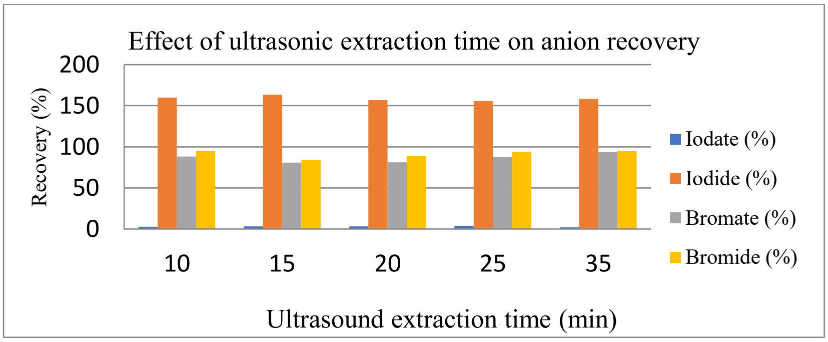

Figure 8. *Results of the investigation of ultrasound extraction time*

The results show that under these conditions, the initial iodate species was almost completely transformed or lost (remaining at only ≈3%), whereas the iodide species increased substantially (≈160%) due to the conversion of iodate through redox reactions with reducing agents present in the sample matrix. These findings further confirm that ultrasound-assisted extraction in water is not feasible for simultaneous speciation analysis, owing to the poor stability of iodate.

Extraction time was subsequently investigated using 10 mM ammonium carbonate containing 0.5 mM Mg-EDTA, combined with horizontal shaking at room temperature, with the aim of achieving adequate recoveries while minimizing iodate-to-iodide interconversion.

The results indicate that horizontal shaking at ambient temperature markedly improved the extraction efficiency of the target anions, particularly iodate (**Figure 9**).

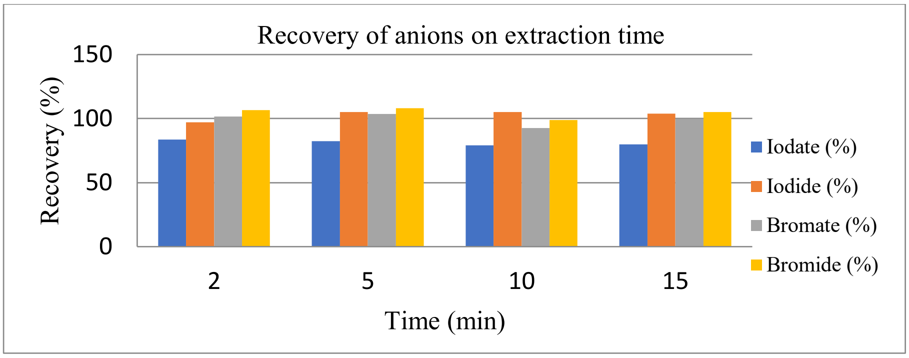

Figure 9. *Investigation of the effect of horizontal shaking extraction time*

While the recoveries of the other anions exhibited no significant changes with increasing extraction time, the iodate recovery showed a slight decrease from 83.5% at 2 min to approximately 79% at 10-15 min. This behavior suggests that iodate is susceptible to transformation during prolonged extraction, most likely being reduced to iodide by reducing substances present in the milk matrix. Therefore, to enable simultaneous speciation of these anions, a short extraction time (approximately 2-5 min) was selected to minimize interconversion and ensure the accuracy of the chemical speciation analysis. The optimized sample analysis procedure diagram is presented in **Figure 10**.

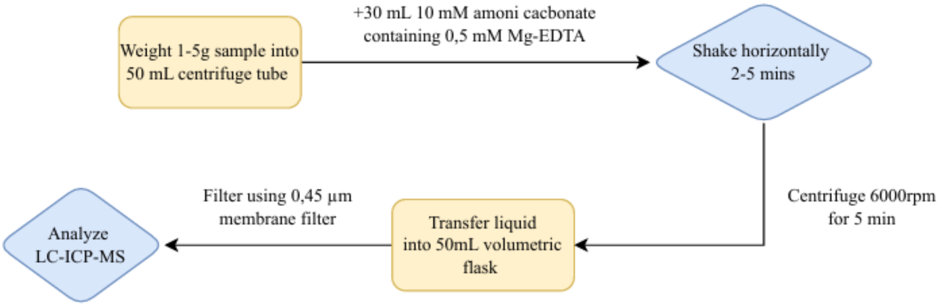

Figure 10. *Diagram of the sample analysis procedure*

3.3. Method validation

3.3.1. Specificity and selectivity

Specificity was evaluated by comparing chromatograms of the blank, the standard solution, and the blank spiked at an equivalent concentration of 20 ppb. As shown in **Figure 11**, no analyte signals were observed in the blank matrix (**Figure 11-A1&A2**), while the spiked blank (**Figure 11-B1&B2**) exhibited peaks at retention times consistent with those of the standards (**Figure 11-C1&C2**), with retention-time deviations not exceeding 2%. These results, together with the use of collision mode to eliminate isotopic interferences, demonstrate that the method provides adequate specificity and selectivity for determining iodide, iodate, bromide, and bromate in milk matrices.

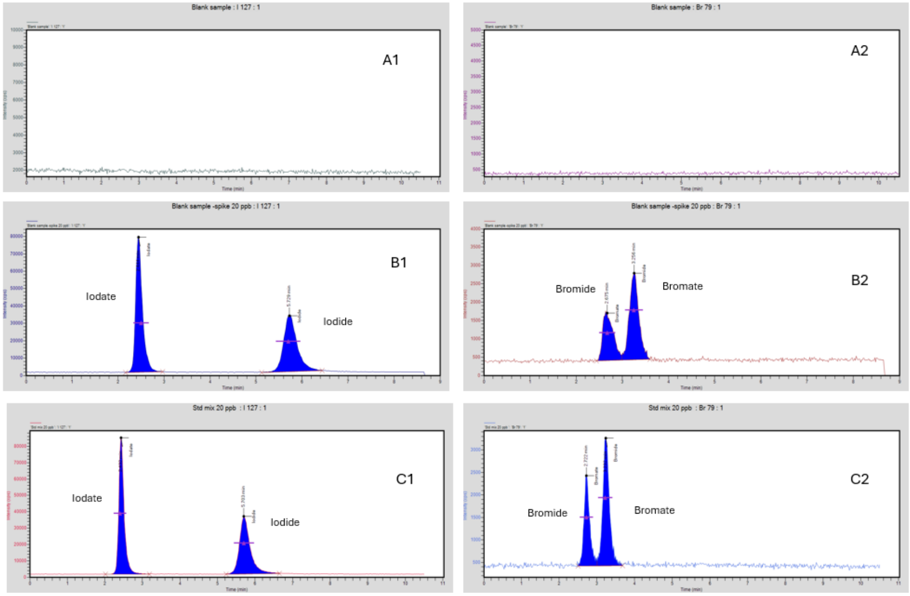

Figure 11. *Results of the specificity and selectivity evaluation (11A – blank sample, 11B – spiked sample, 11C – standard solution)*

3.3.2. Calibration Curve

A series of working standard solutions for iodine (2–100 ppb) and bromine (5–100 ppb) species were analyzed, and the linear dependence of the chromatographic peak area for each analyte on the corresponding concentration was evaluated. The parameters of the resulting working calibration curves are presented in **Table 2.**

Table 2. *Parameters of the analytical calibration curves for iodine and bromine species*

Analyte	Concentration (ppb)	Calibration equation	Determination coefficient R^2
Iodate	2 – 100	$y = 40861x + 70.78$	$R^2 = 0.9999$
Iodide	2 – 100	$y = 35940x - 29014$	$R^2 = 0.9998$
Bromate	5 – 100	$y = 1029x + 873.12$	$R^2 = 0.9991$
Bromide	5 – 100	$y = 1766.3x - 5104.5$	$R^2 = 0.9996$

The results indicated that the working calibration curves for all analytes exhibited correlation coefficients $R^2 \geq 0.995$. The accuracy of the working standard points was ≤ 20% at its lowest value, which is acceptable for quantitative analysis at the LOQ level. Therefore, these working ranges of the calibration curves satisfied the requirements for practical application in real sample analysis.

3.3.3. Limit of detection and limit of quantification

The LOD and LOQ were determined by spiking gradually decreasing concentrations of the analyte mixture into the blank sample (a test sample where the analytes were not detected) until a signal-to-noise ratio (S/N) of 10 was obtained for the LOQ value, and a ratio of S/N = 3 was obtained for the LOD value.

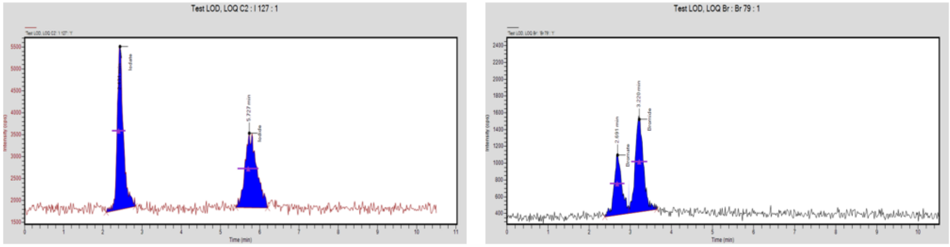

Figure 12. *Chromatograms of iodine and bromine species at the LOQ level*

Experimental results indicated that the limits of quantification (LOQ) for the analytes in liquid and solid matrices were as follows, respectively: iodate: 1.10 and 5.50 µg/100g; iodide: 1.77 and 8.90 µg/100g; bromate: 8.50 and 42.5 µg/100g; and bromide: 12.2 and 61.0 µg/100g.

3.3.4. Precision and accuracy of the method

The precision of the method was assessed through repeatability, while trueness was evaluated based on recovery. A liquid milk sample previously characterized to contain iodide and bromide at levels below the limit of quantification-and in which iodate and bromate were not detected-was selected for the validation experiment. The sample was spiked with a mixed standard solution of the four target anions at an intermediate concentration level of 20 µg/100g and analyzed in six replicates under the optimized conditions.

The results showed that the repeatability (%RSD) for all four analytes ranged from 1.90% to 6.01%, and recoveries were between 86.3% and 109.8%. These values comply well with AOAC performance criteria for quantitative analysis (repeatability < 11% and recovery within 80–110%). Therefore, the method was validated as suitable for the quantitative determination of these analytes in real milk samples.

3.3.5. Analysis of real samples

Based on the established and validated method, the concentrations of the different iodine- and bromine-containing species were determined in commercially available food samples, including powdered milk (n = 9), liquid milk (n = 12), and yogurt products (n = 5) collected randomly across the Hanoi area (**Figure 13**). The analytical results revealed distinct distribution patterns of iodine and bromine species among the three product categories.

Notably, iodate and bromate were not detected in any of the analyzed samples. This finding is consistent with the chemical characteristics of these species as well as practical manufacturing practices, and aligns with the results reported by Lawrence P. *et al.* [9]. Iodate is known to be unstable in milk matrices, especially under reducing conditions or in acidic environments such as yogurt, where it is readily converted to iodide [25]. In dairy processing and production of nutritional products, iodine is typically added in the form of iodide due to its high solubility and superior bioavailability [3]. Similarly, bromate-being strictly prohibited as a food additive because of its carcinogenic potential-was absent in all samples, reflecting compliance with food safety regulations by the manufacturers.

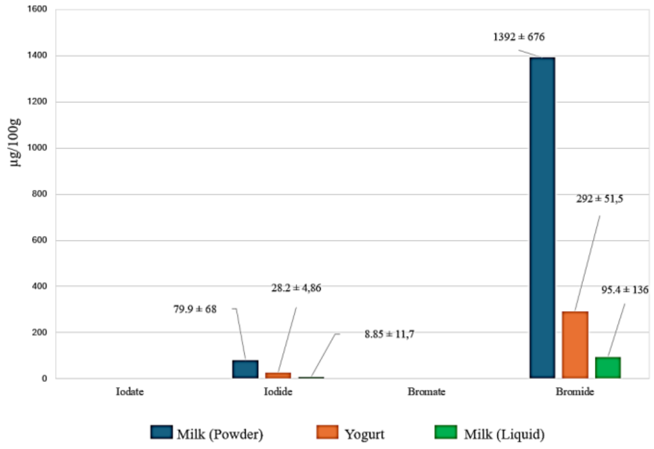

Figure 13. *Average content of iodine and bromine species in milk products ($X_{tb} \pm \sigma$)*

For iodide, its presence was observed across all three product categories, albeit with distinct distribution patterns. Iodide was detected in every powdered milk sample, with concentrations spanning a wide range from 13.5 to 203 µg/100g. In liquid milk, iodide was also found in most samples (10 out of 12), though at substantially lower levels (1.29–34.4 µg/100g). In contrast, yogurt products exhibited a narrower and more consistent concentration range, with all five samples containing iodide between 20.5 and 32.0 µg/100g.

Regarding bromide, this species was detected at varying levels in all three product groups. Powdered milk contained the highest concentrations, ranging from 996 to 2229 µg/100g (equivalent to 10–22 mg/kg). Liquid milk also showed the presence of bromide in several samples but at markedly lower concentrations (5.6–397 µg/100g, approximately 0.06–4 mg/kg), while yogurt samples contained intermediate levels (259–383 µg/100g, approximately 2.6–3.8 mg/kg). The occurrence of bromide in most samples is likely attributable to its natural presence in milk or trace contributions from raw materials, rather than intentional addition or residues from methyl bromide used as a fumigant, since its application in food sterilization and preservation has been banned in many countries due to environmental and health concerns [30]. Nevertheless, the presence of bromide-particularly in powdered milk products-may pose nutritional and health implications, as elevated bromide levels can interfere with iodine uptake and potentially increase the risk of hypothyroidism [31].

4. CONCLUSION

This study successfully developed and optimized an LC-ICP-MS method for the simultaneous determination of iodine species (iodide and iodate) and bromine species (bromide and bromate) in milk matrices, achieving repeatability (1.90–6.01%) and recoveries (86.3–109.8%) compliant with AOAC performance criteria. Analysis of 26 milk samples collected across Hanoi revealed that iodate and bromate were not detected in any sample, whereas iodide was present at higher concentrations in powdered milk (13.5–203 µg/100g) compared with yogurt products (20.5–32.0 µg/100g) and liquid milk (1.29–34.4 µg/100g). Bromide was also widely detected, ranging from 5.6 to 2229 µg/100g, with the highest levels observed in powdered milk. These findings confirm the reliability of the LC-ICP-MS method and provide valuable data for assessing the quality of iodine-fortified products as well as potential food safety concerns related to bromine exposure in dairy products.

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